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Future Directions—Toxicology Studies of 1,3-Butadiene and Isoprene

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Examination of a profile of data already existing on 1,3-butadiene shows adequate knowledge in many areas of toxicology that are conventionally required in hazard identification. However, while much progress has been made in areas of metabolism and pharmacokinetics, further studies would be worthwhile to improve mechanistic understanding such as the examination of alternate metabolic pathways, the generation of interspecies scaling factors, and an assessment of the relevance of various tumor sites. In this respect, data pertaining to repeated and pulse exposures of rodents and primates would be helpful.

Another important aspect is the need to understand any human health implications of the observed 1,3-butadiene-induction of the murine leukemia virus. In this respect, studies have been pursued that include the comparison of leukemogenesis in congenetic strains, the leukemogenicity of viral isolates in rodent carcinogenicity and human cell culture studies, and the mechanisms of activation of ecotropic proviral sequences.

Molecular epidemiological and toxicological research is ongoing in rodents and primates to evaluate hemoglobin adduct formation as an index of 1,3-butadiene exposure. Challenges of specificity, sensitivity, and simplification of current procedures need to be overcome.

Recent mutagenicity and metabolism data suggest that structurally-related isoprene may have carcinogenic potential. The use of interstrain comparative studies and data in a second species is discussed, as well as proposed metabolism studies.

Review of 1,3-Butadiene Toxicity

In discussing future research directions for 1,3-butadiene it is appropriate to briefly review the current status of toxicity information on 1,3-butadiene. Existing animal toxicity data for 1,3-butadiene can be arrayed as a toxicological profile in the format that has been utilized by the Agency for Toxic Substances and Disease Registry for other chemicals (Fig. 1). From this, it is apparent that adequate data exist for these conventional toxicity fields (acute, chronic etc.) to characterize toxic potential, particularly by the inhalation route. There are data in other fields too. With respect to mutagenicity, until recently 1,3-butadiene *per se* had only been tested in the Salmonella/Ames assay (*1*). Now, *in vivo* genotoxicity data has been generated by Tice et al. (*2*) and Cunningham et al (*3*). The latter authors show an interesting species difference in the incidence of sister chromatid exchanges and of micronuclei occurring in the bone marrow of the mouse but not of the rat. Sister chromatid exchange in the peripheral blood of 1,3-butadiene-exposed primates is being evaluated. Finally, immunotoxicity has been adequately studied by Thurmond et al. (*4*) with overall no immunological effect seen.

Possible Research Areas

Three areas for possible research are *a*) metabolism and pharmacokinetic studies (repeated and pulse exposure in rodents and primates); *b*) mechanisms of murine leukemogenesis (rodent carcinogenicity and human cell culture studies), *c*) hemoglobin adduct formation as a means of biological monitoring. Each of these areas is now discussed in turn.

Metabolism and Pharmacokinetic Studies

Bolt et al. (*5*) and Kreiling et al. (*6*) have shown that 1,3-butadiene is metabolized to 1,2-epoxy-3-butene both

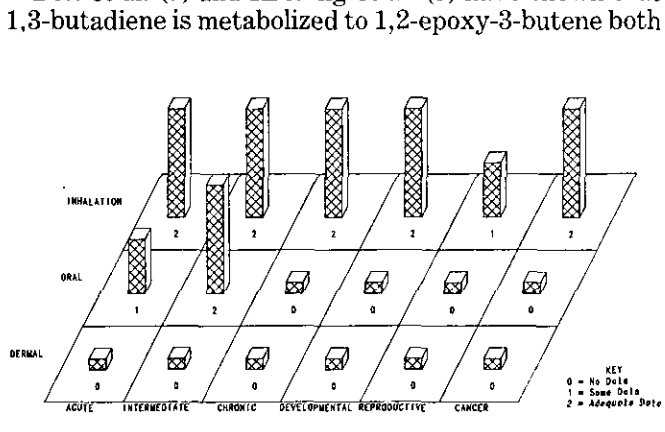

FIGURE 1. Adequacy of the data base on health effects of 1,3-butadiene.

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in vitro and *in vivo*. Bond et al. (*7*) have confirmed and extended these observations by demonstrating that the mutagenic 1,2-epoxy-3-butene and diepoxybutane are present in the blood as metabolites of 1,3-butadiene and that these epoxides have been detected at two to five times greater concentrations in the blood of mice than of rats (*8*).

The species differences and variety of carcinogenic and hematological responses indicated in the rodent carcinogenicity studies of 1,3-butadiene (*9,10*) cast doubt on the omnipotent role presently attributed to these epoxides and allows for the presence and toxic significance of other metabolites. In addition, observations by Anderson et al. (*11*) show a lowering of hepatic nonprotein sulfhydryl levels following the IP injection of 1,2-epoxy-3-butene, and suggest that the epoxide had been further metabolized. Glutathione *S*-transferase may play an important role in a toxification or a detoxification pathway for 1,3-butadiene metabolism in the primate (Fig. 2). Further comparative species studies of the rate and extent of glutathione conjugation and of epoxide hydrolase activity would be beneficial in that they might supply further reason for the species differences in sensitivity or tissue susceptibility.

One possibility that might explain some of the acute hematological effects seen with 1,3-butadiene is the hypothesis that 1,3-butadiene can be metabolized in the mouse to the homologous 4-carbon dialdehyde, namely butenedial (*12*). In a joint collaborative project with Surrey University (UK) and the Robert Wood Johnson Medical School in New Jersey, computer graphics and electronic structure calculations (Tables 1 and 2) were used to determine that butenedial is likely to share similar lipophilicity and toxic properties as of the 6-carbon dialdehyde, muconaldehyde. Muconaldehyde has been shown to produce hematotoxicity (*13*).

Although it is now known that 1,3-butadiene does not induce its own metabolism (*15*), further pharmacokinetics and metabolism studies using pulse exposures and

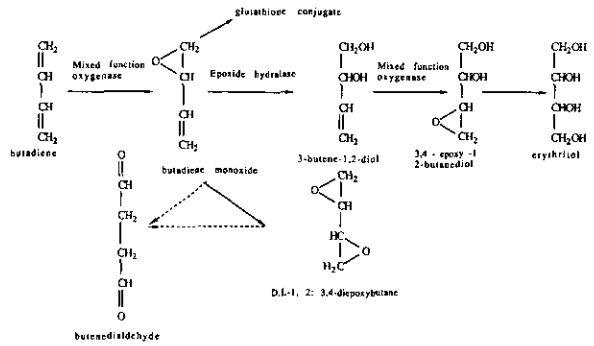

FIGURE 2. Potential pathway for the formation of butenedialdehyde.

also repeated exposures, rather than single (6-hr) ones, may contribute to our mechanistic understanding of 1,3-butadiene toxicity. It is possible that such exposures may affect a quantitatively minor metabolic pathway, which has major toxicological ramifications. These studies may elucidate why exposure concentration is a more important determinant in thymic lymphoma/leukemia formation than the duration of exposure. The data could also be used in developing scaling factors essential for interspecies extrapolation of risk assessment. Interspecies disposition studies involving DNA adduct determinations in target tissues may provide answers as to the reason for the wide range of tumor sites observed in the mouse compared to those in the rat.

Mechanisms of Murine Leukemogenesis

The studies of Irons and coworkers (*16,17*) show that the endogenous retrovirus (MuLV) may play a role in 1,3-butadiene-induced T-cell lymphoma. A marked difference was found between the incidence of thymic lymphoma/leukemia in the B6C3F$_1$ mouse (57%) exposed to 1,3-butadiene for 1 year and the incidence in the similarly exposed NIH Swiss mouse (14%), a strain in which the MuLV proviral sequences are incomplete. As a future research direction, Irons has suggested that the

Table 1. MINDO/3 optimized molecular dimensions for dialdehydes.[a]

Compound	Length, Å	Width, Å	Depth, Å	Area/depth	Area/depth^2	Length/width
Muconaldehyde	10.3	5.5	4.9	11.6	2.4	1.9
Butenedialdehyde	8.1	5.5	3.3	13.5	4.1	1.5

[a]Calculated by MINDO/3 method of Bingham et al. (*14*).

Table 2. Electronic parameters for dialdehydes.[a]

Compound units	E (HOMO)[b] eV	E (LEMO)[b] eV	ΔE[c] eV	SE[d] electrons/eV	QT[e] e	μ[f] D
Muconaldehyde	− 9.85	−0.52	9.33	2.68	2.74	0.00
Butenedialdehyde	−10.04	−0.47	9.57	2.01	2.44	0.00

[a]Calculated by MINDO/3 method of Bingham et al. (*14*).
[b]E (HOMO) and E (LEMO) are the highest occupied and lowest empty molecular orbital energies, respectively, in electron volts (eV).
[c]ΔE is the difference between E (HOMO) and E (LEMO) energies in electron volts (eV).
[d]SE is the electrophilic superdelocalizability summed over all the atoms in electrons per electron volt.
[e]QT is the net atomic charge summed over all atoms in electron units (e).
[f]μ is the dipole moment in Debyes (D).

significance of the B6C3F$_1$ mouse as a model for the assessment of leukemia risk in man should be reexamined. The research he envisages would include comparative incidence studies on congenic mouse strains (selected so that any difference in strain response to 1,3-butadiene exposure would be attributable only to the retroviral gene differences), and a study of the leukemic potential of viral isolates from 1,3-butadiene-induced tumors. Other studies would include the use of *in vitro* culture systems using both human and murine cells to determine whether or not 1,3-butadiene and its metabolites can alter retroviral expression in the mouse and, similarly, in human cells. Primate studies (with the Simian monkey) could be used for further study of retroviral interaction and of bone marrow changes. Such studies may contribute to our understanding of the relevance of the murine response for other chemicals in addition to 1,3-butadiene.

Hemoglobin Carcinogenicity and Human Cell Culture Studies

The final area is that of adduct formation. Laib (*18*) has reported the detection of a DNA adduct with 1,2-epoxy-3-butene and other uncharacterized adducts in the blood of mice exposed to 1,3-butadiene. NIEHS is working on the use of hemoglobin adducts as biological markers of exposure. Dahl (*15*) reported a dose-related covalent binding of 1,3-butadiene to hemoglobin in the rodent and the presence (though at near detection limits) of similar adducts in the primate at the one concentration of 1,3-butadiene (10 ppm in air) examined to date. The next stage is to refine the sensitivity and specificity of the assay and to switch from the use of radiolabel to a cold method.

Further research on the toxicology of isoprene is also needed since this chemical is structurally similar to 1,3-butadiene, and recently reported (*19*) micronucleus and sister chromatic exchange studies indicate that isoprene has some mutagenic potential in mice. Studies by Dahl et al. (*20*) and by Del Monte et al. (*21*) indicate that the metabolism of isoprene may be qualitatively similar to that of 1,3-butadiene in terms of the formation of equivalent mono- and diepoxides. Studies of isoprene in primates are being performed to characterize the metabolic profile in that species following an increasing series of concentrations each of 2 hr duration. Melnick et al. (*22*) are using an extended 3-month toxicity study (6-month exposure/6-month hold) to determine whether or not isoprene will produce thymic lymphoma/leukemia and other tumors in the B6C3F$_1$ mice, which was the case for 1,3-butadiene under a similar testing regimen. Further metabolism studies in the B6C3F$_1$ mouse are also planned by the National Toxicology Program to compliment the published work in the rat.

The International Institute of Synthetic Rubber Producers (IISRP) is examining the merits of a 1-year interstrain comparative study of isoprene, similar to that conducted by Irons (*16*) with 1,3-butadiene in the NIH Swiss and B6C3F$_1$ mouse and a two-year study of isoprene in the Sprague-Dawley rat. These studies would provide a comparison to 1,3-butadiene and complement the possible retrovirus research proposal, described previously for 1,3-butadiene. Industrial hygiene data-gathering and a prospective epidemiology study are also being considered for isoprene.

Conclusion

Our understanding of 1,3-butadiene, more so than for many other compounds, has benefitted from the recent burst of research activity on this compound. It is anticipated that future research will lead to a better understanding of species differences in metabolism and tumorigenic response, which in turn should reduce current uncertainties in risk-assessment modeling.

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